[CANCER RESEARCH 50, 4366–4370, July 15, 1990]

Interspecies Scaling of the Pharmacokinetics of *N*-Nitrosodimethylamine[1]

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ABSTRACT

The pharmacokinetics of *N*-nitrosodimethylamine was studied in patas monkeys following i.v. doses of 0.5, 1.0, and 5.0 mg/kg and a p.o. dose of 1.0 mg/kg, and in Swiss mice at i.v. doses of 1.0 and 2.0 mg/kg. In the patas monkey the pharmacokinetics was linear over the i.v. dose range studied. The mean clearance (Cl), steady-state volume of distribution (V_{ss}), mean residence time, and elimination half-life ($t_{½}$) were 103.3 ± 26.7 (SD) ml/min, 3061 ± 821 ml, 30.8 ± 10.8 min, and 21.1 ± 8.5 min, respectively. Assuming that the pharmacokinetics was linear at the p.o. dose used, the p.o. bioavailability of *N*-nitrosodimethylamine in the monkey was 49%. The pharmacokinetics was also linear in mice, and the average Cl, V_{ss}, mean residence time, and $t_{½}$ were 3.81 ml/min, 21.0 ml, 5.5 min, and 11.9 min, respectively. These data and data for rats, hamsters, rabbits, dogs, and pigs taken from the literature were used to scale Cl and V_{ss} to body weight using the allometric equation. The resulting equation for Cl was $Cl = 49.7B^{0.998}$ and the equation for V_{ss} was $V_{ss} = 748B^{1.05}$ where B is body weight in kg. The fit of the data to the equation was excellent in both cases. Using these equations and assuming a body weight of 70 kg for humans, the Cl and V_{ss} for *N*-nitrosodimethylamine in humans are estimated to be 3450 ml/min and 64,800 ml, respectively.

INTRODUCTION

Attempts to assess the risk posed to humans by chemical carcinogens are usually based on studies performed in rodents exposed to relatively high doses of the carcinogen for a substantial fraction of their life span (1). The data are then extrapolated to lower doses to which humans may be exposed for an undetermined period of time. The accuracy of this process depends on knowledge and understanding of the ways in which the animal models resemble and differ from the human with regard to the various parameters that determine or modify the effect of carcinogens. These parameters include a wide range of phenomena from disposition and clearance (pharmacokinetics) at the whole animal level to enzymatic activation and macromolecular alterations and repair in the cell.

At the present time, pharmacokinetics is a particularly attractive factor to study in this regard, since it has become increasingly apparent that the qualitative and quantitative impact of a carcinogen may be profoundly altered simply by changing its rate of clearance and pattern of distribution (2–4). This implies that analysis and modulation of the pharmacokinetics of cancer-causing chemicals in the human could contribute in an important way to both risk assessment and risk management.

Can the pharmacokinetics of carcinogens in humans be understood on the basis of extrapolation from animal models? Workers have started to address this question using a representative of an important class of environmental carcinogen, NDMA.[4] Nitrosamines, especially NDMA, occur widely in human exposure sources, form *in situ* by nitrosation and transnitrosation of amines and amides, and are frequently detected in low concentrations in human tissues (5–7). Also there is evidence that alterations in the pharmacokinetics of NDMA by suppression of hepatic metabolism permits or increases tumorigenicity at extrahepatic sites (8–12). Understanding the relevance of such pharmacokinetic phenomena for the human is important, and the data presented here suggest that it may be at least partially attainable by the experiments involving interspecies comparisons.

In our laboratories and those of others, data have been accumulating on the pharmacokinetics of NDMA in the hamster (13), rat (12, 13), rabbit (14), dog (15), and pig (16). In this paper, data for the mouse and patas monkey are added to yield data for a total of seven species ranging widely in size and representing phylogenetically and physiologically disparate organisms. Allometric analysis, a process which correlates a physiological function or condition with body weight, was then undertaken with these data. Several authors have shown for a variety of chemical compounds that pharmacokinetic parameters such as half-life, clearance, intrinsic clearance, and volume of distribution varied simply as a function of body weight (17–24). Thus, in double-log plots, these parameters varied linearly with weight for species ranging in weight from 30 g to 70 kg. The potential for extrapolation to the human was evident when data from a sufficient number of species varying widely in body weight were available.

In performing the same interspecies scaling of pharmacokinetic parameters for NDMA in seven species, a consistent pattern emerged for this compound, with some interesting comparative differences in variation of clearance and bioavailability. The results are potentially extrapolatable to humans and have provocative implications regarding the potential biological effects of this chemical.

MATERIALS AND METHODS

Chemicals. NDMA was obtained from Sigma Chemical Co. (St. Louis, MO). Antifoam B was acquired from Fisher Scientific Co. (King of Prussia, PA). Morpholine (Aldrich Chemical Co., Milwaukee, WI) was double distilled and stored under nitrogen. All other chemicals were ACS reagent grade or better.

Pharmacokinetics of NDMA in Mice. Male Swiss mice [(CR:NIH(s))], 6–8 weeks old with an average weight of 27 g were obtained from the Animal Production Area of the Frederick Cancer Research Facility. The mice were housed under pathogen-free conditions, and hardwood shavings were used as bedding. Conditions included a 12/12-h fluorescent light/dark cycle, the temperature was maintained at 24 ± 2°C, and humidity at 55 ± 5% (SD). The diet consisted of NIH 31 Open Formula

Received 12/7/89; revised 3/26/90.

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[1] This study was supported in part by USPHS Grant CA43342 by the National Cancer Institute, Department of Health and Human Services.

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[4] The abbreviations used are: NDMA, *N*-nitrosodimethylamine; $AUC_{p.o.}$, area under the orally dosed blood concentration *versus* time curve; $AUC_{i.v.}$, area under the i.v. dosed blood concentration *versus* time curve; Cl_s, systemic clearance from blood; Cl_{int}, intrinsic clearance; Q_H, hepatic blood flow; V_{ss}, steady state volume of distribution; F, oral bioavailability; ER, hepatic extraction ratio.

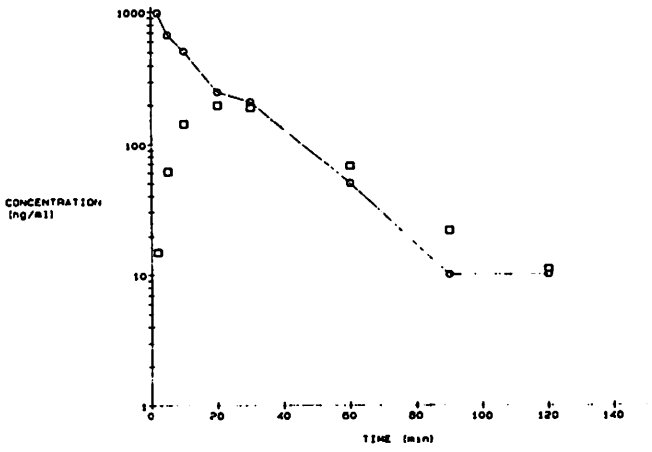

Fig. 1. Typical blood concentration *versus* time profile of NDMA given to monkey R160 at a dose of 1.0 mg/kg both i.v. bolus (O) and orally (□).

Autoclavable Diet and acidified water. NDMA was dosed via the tail vein after warming either the whole animal or just the tail. Pooled blood, 15 mice/time point, was collected in heparinized tubes at specific time intervals by decapitation.

Pharmacokinetics of NDMA in Monkeys. Colony-reared male patas monkeys, weighing 2–4 kg (2–4 years old) were maintained on Purina monkey chow supplemented by fresh fruit and vegetables. Prior to treatment, they were trained to accept restraint and needle insertion without distress, so that treatment could be accomplished without either anesthesia or discomfort to the animal. NDMA was administered via the saphenous vein or by gavage. Blood samples were taken from the femoral vein and placed in heparinized tubes.

Determination of NDMA in Blood. The concentration of NDMA in blood was determined by the method described by Pylypiw *et al.* (25). Briefly, 2 ml of blood, concentrated sulfuric acid, sulfamic acid, and Antifoam B in precise ratios were extracted using methylene chloride with a specially modified extraction-distillation apparatus (11). The samples were then concentrated and analyzed by gas chromatography-thermal energy analyzer. A model TEA-502 thermal energy analyzer (Thermo-Electron Corp., Waltham, MA) was put in series with a HP 5791A packed column gas chromatograph (Avondale, PA).

Pharmacokinetic Calculations. The blood concentration *versus* time data following i.v. dosing were fit to a one or two compartment open model using PHARM, a pharmacokinetic parameter estimation program (26). The goodness-of-fit was determined by visual inspection of both the concentration *versus* time profile and the plot of the residuals. The area under the curve from time zero to the last value was determined using the trapezoidal rule, and the extrapolated area was calculated by dividing the concentration at the final time point by the apparent elimination rate constant. The extrapolated area did not exceed 26% of the total area in any case. The V_{ss}, Cl_t, and mean residence time were determined using noncompartmental methods (27). Bioavailability was determined using Equation A.

$$F = (AUC_{p.o.} \cdot dose_{i.v.})/(AUC_{i.v.} \cdot dose_{p.o.}) \quad (A)$$

Interspecies Extrapolation. With the data obtained in these experiments and those found in the literature, interspecies scaling plots were made. By using the allometric equation

$$y = aB^x \quad (B)$$

where B is body weight and x and a are the allometric exponent and coefficient, respectively, an equation can be obtained in which the body weights of different species can be related to physiological parameters shared by that group. By plotting a specific parameter as a function of body weight on a log-log plot, a line can be fitted to the points with acceptable correlation. The parameters a and x were estimated by performing a weighted ($1/y^2$) nonlinear regression analyses using RS/E (BBN Software, Cambridge, MA).

RESULTS

In the monkey, NDMA concentrations decreased monoexponentially after an i.v. bolus dose (Fig. 1). The AUCs were roughly proportional to the dose which suggests that active processes were not saturated over this dose range (Table 1). Therefore, Cl, V_{ss}, and mean residence time were not dose related and had values of 103.3 ± 26.7 ml/min, 3061 ± 821 ml, and 30.8 ± 10.8 min, respectively. The mean half-life of NDMA in blood was 21.1 ± 8.5 min.

Following a p.o. dose of 1.0 mg/kg, the concentrations of NDMA in blood increased reaching an average C_{max} of 205 ng/ml 25 min after dosing (Fig. 1; Table 2). The concentration then decreased monoexponentially with an average half-life of 22.9 min. Since this half-life is similar to the elimination half-life observed after i.v. administration, it represents an elimination half-life and is not absorption limited. The p.o. bioavailability of NDMA, assuming linear pharmacokinetics, was estimated to be 49%.

In mice, NDMA concentrations decreased biphasically after various i.v. bolus doses (Fig. 2) except in experiment 2 which exhibited a monoexponential decrease in the concentration *versus* time profile. The mice had an average elimination half-life of 11.9 min and a distribution half-life of 3.2 min (Table 3). The average clearance in mice was 3.81 ml/min, and the average V_{ss} was 21.0 ml.

To determine if the pharmacokinetic parameters for NDMA vary predictably with body weight, data from the present study and from the literature were pooled (Table 4) and Cl (Fig. 3)

Table 1 *Pharmacokinetic parameters for NDMA administered i.v. to monkeys*

Patas monkeys were dosed i.v. with 0.5, 1.0, or 5.0 mg/kg NDMA via the saphenous vein. Heparinized blood samples from the femoral vein were analyzed in order to determine the pharmacokinetic parameters.

Animal	Body wt (kg)	AUC (min·ng/ml)	Cl (ml/min)	V_{ss} (ml)	Mean residence time (min)	$t_{½}$ (min)
			0.5 mg/kg			
R165	2.34	11,949	97.8	2,242	22.9	15.8
R161	2.60	13,068	100.0	2,954	29.7	20.7
			1.0 mg/kg			
R160	2.68	17,810	149.2	3,322	22.3	14.2
			5.0 mg/kg			
R163	1.80	110,365	81.5	2,464	30.2	19.2
R162	2.95	166,973	88.2	4,322	48.9	35.5
Overall mean ± SD	2.47±0.43		103.3±26.7	3,061±821	30.8±10.8	21.1±8.5

Table 2 *Pharmacokinetic parameters for N-nitrosodimethylamine administered p.o. to monkeys*

Patas monkeys were dosed p.o. by gavage with 1.0 mg/kg NDMA. Bioavailability was calculated using 0.5 mg/kg i.v. data.

Animal	AUC (ng/ml·min)	C_{max} (ng/ml)	t_{max} (min)	$t_{½}$ (min)	Mean residence time (min)	F (%)
R160	9,871	199	20	21.9	42.3	40
R178	14,014	211	30	23.9	51.9	57
Av.	11,943	205	25	22.9	94.2	49

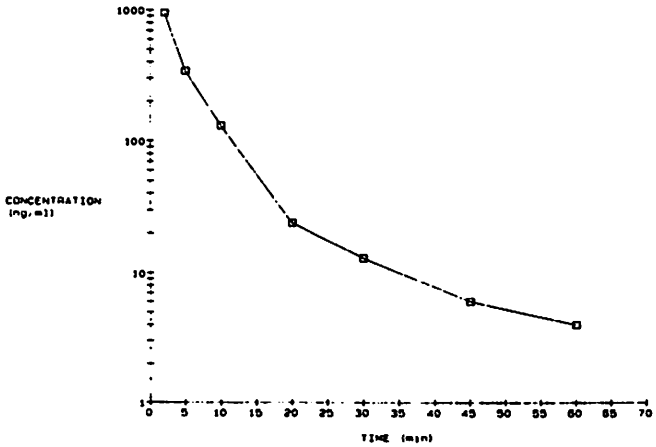

Fig. 2. Typical blood concentration *versus* time profile of NDMA given to mice at 1.0 mg/kg i.v. Individual *points* represent the concentration of pooled samples.

Table 3 *Pharmacokinetic parameters for N-Nitrosodimethylamine administered i.v. to mice*

Male Swiss mice were dosed i.v. via the tail vein with two different doses of NDMA.

Experiment	AUC (ng/ml·min)	Cl (ml/min)	V_{ss} (ml)	Mean residence time (min)	$t_{½}\alpha$ (min)	$t_{½}\beta$ (min)
		1.0 mg/kg				
1	6,773	3.69	24.1	6.5	2.5	15.4
2	7,615	3.28	17.4	5.3	4.1	
		2.0 mg/kg				
3	11,250	4.45	21.5	4.8	3.1	8.3
Mean		3.81	21.0	5.5	3.2	11.9

Table 4 *Pharmacokinetic parameters for NDMA in several animal species*

Species	Body wt (kg)	Q_H[a] (ml/min)	Cl (ml/min)	V_{ss} (ml)	F (%)	Ref.
Mouse	0.025	2.0	3.8	21.0	ND[b]	
Hamster	0.112	7.8	5.6	70.6	11	13
Rat	0.200	13.1	8.0	50.5	8	12, 13
Rabbit	2.5	126	163.0	1,358.0	ND[b]	14
Monkey	2.5	126	103.3	3,061.0	49	
Dog	13.0	549	608.0	22,800.0	93	15
Pig	40.0	1,499	2,516.0	40,000.0	67	16

[a] Hepatic flow rates were calculated from the equation $Q_H = 0.055 B^{0.894}$ (23).
[b] ND, not done.

and V_{ss} (Fig. 4) were fit to the allometric equation. The equations resulting from a nonlinear regression fit are

$$Cl = 49.7(\pm 5.25)B^{0.998(\pm .049)}$$
$$V_{ss} = 748(\pm 116)B^{1.05(\pm .073)}$$

where B is body weight and the allometric parameters are given as the point estimate ± SE.

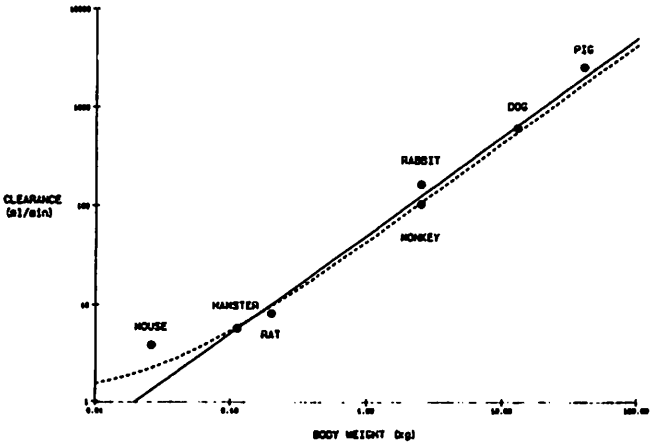

Fig. 3. Interspecies scaling of systemic NDMA clearance as a function of body weight as determined from a weighted ($1/y^2$) nonlinear regression using the allometric equation (——) and from a weighted ($1/y^2$) linear regression (– – – –) presented on a log-log plot. The 95% confidence interval for the allometric equation ($Cl = aB^x$) are $36.2 < a < 63.2$ and $0.872 < x < 1.12$. The 95% confidence interval for the parameters of the linear equation ($Cl = aB + C$) are $24.6 < a < 59.6$ and $-1.03 < C < 3.23$.

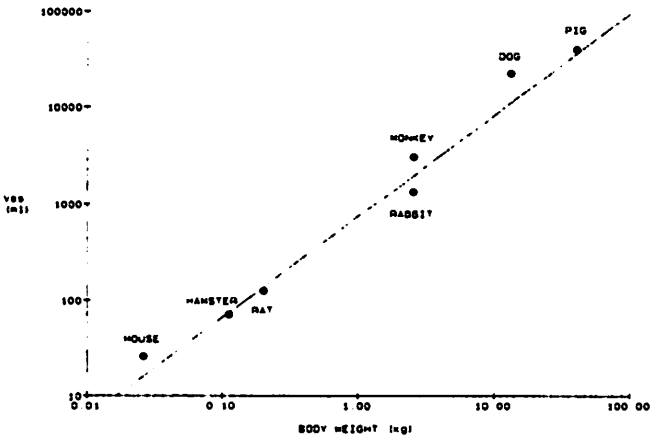

Fig. 4. Interspecies scaling of the steady state volume of distribution of NDMA as a function of body weight. The 95% confidence interval of the parameters from the allometric equation ($V_{ss} = aB^x$) are $450 < a < 1045$ and $0.866 < x < 1.24$.

Since the exponent for clearance was extremely close to 1, a weighted ($1/y^2$) linear regression was performed. The equation obtained from this fit is

$$Cl = 42.1B + 1.10$$

Assuming a body weight of 70 kg for humans, estimates of Cl and V_{ss} for humans from the allometric equation are 3,450 ml/min and 64,800 ml, respectively. An estimate of clearance in humans using the linear equation is 2950 ml/min.

DISCUSSION

The role that the pharmacokinetics of a carcinogen plays in its impact, both qualitatively (*i.e.*, target organ) and quantitatively (*i.e.*, risk assessment), has not been adequately determined for most compounds assumed to be or suspected to be human carcinogens. Data from animals are used to assess the risk based on dose (in mg/kg) without regard for differences in the rate of elimination (clearance) or distribution of the compound between species. It is clear that these factors play a role since, for carcinogens such as the nitrosamines, the route of administration can alter the organospecificity as can manipu-

lation of the clearance with inducers or inhibitors of metabolism. The ultimate question is: what is the pharmacokinetic profile of a given carcinogen in humans? It is obviously unethical to perform an experiment to answer this question, but it may be possible to obtain information that could be useful in estimating the risk to humans without direct measurement in humans. It has been shown for several drugs that key pharmacokinetic parameters such as Cl, Cl_{int}, and V_{ss} can be scaled to body weight using the allometric equation (Equation B) (17–24). An interesting generalization from studies that have been done is that, for compounds cleared primarily by metabolism, the allometric exponent is usually less than 1. That is, larger species tend to have lower clearance than smaller species. This is true of many physiological parameters as well (17). Most active processes tend to be slower in large, long-lived species compared to small, short-lived species. The use of carcinogenicity data obtained in small species (rodents) to estimate risk in larger species (humans), which do not take these differences into account, may introduce an error.

We have attempted this type of analysis with the well-known carcinogen NDMA. It is well established that NDMA must be metabolized to the ultimate methylating species to exert its toxic effect. We collected pharmacokinetic data on NDMA in several species and together with data generated in other laboratories determined the fit of the parameters Cl and V_{ss} to the allometric equation. The fit in both cases was excellent.

An interesting observation, however, was that the allometric exponent for both parameters was close to unity. Therefore the correlations were, in fact, linear; thus risk assessment using an extrapolation based on dose (on a mg/kg basis) appears to be justified for NDMA. Exponents of 1 are expected for the volume of distribution, since V_{ss} is a result of a passive process and total body water scales to a value that is also close to unity (19). However, in many cases, clearance due to metabolism, an active process, scales to an exponent of approximately 0.75 which may reflect a relationship of hepatic blood flow to body weight (22).

In spite of good correlations between body weight and both clearance and V_{ss}, there was not a uniformly predictable relationship between body weight and bioavailability. In general, the smaller species tended to show lower bioavailability than the larger species. If it is assumed that NDMA is cleared solely by hepatic metabolism, bioavailability (F) will ultimately depend on Cl_{int} and hepatic blood flow (Q_H). For a compound that is completely absorbed $F = 1 - ER$ where ER is the hepatic extraction ratio, and $ER = Cl_H/Q_H$, and Cl_H depends upon Cl_{int} and Q_H as shown in Equation C.

$$Cl_H = (Q_H * Cl_{int})/(Q_H + Cl_{int}) \quad \text{(C)}$$

If Cl_{int} and Q_H scale differently across species, F will not be a constant function of body weight. In situations where intrinsic clearance greatly exceeds blood flow, then hepatic clearance is blood flow limited and its extraction ratio, and hence bioavailability, should remain constant.

The wide interspecies difference in bioavailability of NDMA is difficult to explain. Compounds with large extractions ratios (>0.8) often show variation in bioavailability on the order of 2-fold, because small changes in ER result in large changes in F. In the case of NDMA, the difference in F between species is about 12-fold despite high clearance in all species. Some other factors that commonly complicate bioavailability determination, including absorption from the gastrointestinal tract and effects due to protein binding and RBC association, do not pertain to NDMA, so an explanation of the anomalous bioavailability data must be sought elsewhere. Most likely, the assumption that the liver is the only clearing organ is incorrect. This interpretation was also supported by the results of an attempt to calculate intrinsic clearance using Equation C.

In all but two cases (rat and monkey), convergence of the iterative process used for solving the equation for Cl_{int} could not be achieved (TK-Solver; Software Arts, Wellesley, MA). For this to occur, the actual hepatic flow rate was lower than that needed to solve the equation; thus, Cl_{int} is blood flow limited and extrahepatic metabolism must occur.

The same conclusion is suggested by the constancy of the systemic clearance per unit body weight among the species, in spite of the apparent wide variation in liver extraction ratios. Possible clearance mechanisms in addition to hepatic metabolism would be excretion of unchanged NDMA in urine or expired in air. Urinary excretion of unchanged compound is minimal, at least in rats, dogs, and monkeys, and, in the monkey, little is found in expired air.[5] With regard to metabolism, kidney has the highest NDMA demethylase activity of the extrahepatic organs, and activity has also been measured in lung (28). If the lung were to play a significant role in the clearance of NDMA then bioavailability estimates may be in error, since extraction of i.v. dose across the lung would reduce the effective dose to the liver and inflate the value of F (Equation A). Recent studies using isolated perfused rat and rabbit lungs have, however, failed to demonstrate any clearance by the lung.[5] In any event, the possibility of significant extrahepatic metabolism in the larger species, with concomitant activation of NDMA to a proximate carcinogen has clear public health implications and is worthy of further study.

Despite the fundamental question of NDMA metabolism raised by these parameters, allometric analyses such as these, should allow estimations of the pharmacokinetic parameters for NDMA in humans. Assuming a body weight of 70 kg for humans, the allometrically extrapolated values of Cl and V_{ss} are 3,450 ml/min and 64,800 ml, respectively. An alternative method for calculating hepatic clearance in humans is to determine enzyme kinetic parameters using human microsomes and extrapolate the data from the enzyme level (*i.e.*, Cl_{int}) to the organ level. This type of analysis by Streeter *et al.* (29) resulted in an intrinsic hepatic clearance of 200 ml/min/kg. This high value for intrinsic hepatic clearance should lead to blood flow limitations in the clearing organ since hepatic blood flow in humans is approximately 20 ml/min/kg. The maximum clearance in humans, assuming no lung clearance, would be approximately 5,000 ml/min (70 ml/min/kg).

We recognize that there are many other processes that play important roles in carcinogenicity that may differ between species, and it is an oversimplification to focus solely on the pharmacokinetics, but to base risk on dose alone is also an oversimplification. If pharmacokinetic data are collected in sufficient number of species covering a wide range of body weight, one can determine if there are any systematic differences in these parameters and perhaps modify risk assessment.

ACKNOWLEDGMENTS

The monkeys were housed under AALAC-accredited housing conditions at SEMA, Inc., Rockville, MD, under National Cancer Institute Contract N01-CP-1078.

REFERENCES

1. Menzel, D. B. Physiological pharmacokinetic modeling. Environ. Sci. Technol., *21:* 944–950, 1987.

[5] Unpublished data.

2. Wattenberg, L. W. Inhibition of chemical carcinogenesis. J. Natl. Cancer Inst., *60:* 11–18, 1978.
3. Anderson, L. M., and Seetharam, S. Protection against tumorigenesis by 3-methylcholanthrene in mice by β-napthoflavone as a function of inducibility of methylcholanthrene metabolism. Cancer Res., *45:* 6384–6389, 1985.
4. Young, J. F., and Kadlubar, F. F. A pharmacokinetic model to predict exposure of bladder epithelium to urinary *N*-hydroxyarylamine carcinogens as a function of urine pH, voiding interval, and resorption. Drug Metab. Dispos., *10:* 641–644, 1982.
5. Poirier, S., Ohshima, H., De-Thé, G., Hubert, A., Bourgade, M. C., and Bartsch H. Volatile nitrosamine levels in common foods from Tunisia, South China, and Greenland: high-risk areas for nasopharyngeal carcinoma (NPC). Int. J. Cancer, *19:* 293–296, 1987.
6. Garland, W. A., Kuenzig, W., Rubio, R., Kornychucjk, H., Norkus, E. P., and Conney, A. H. Urinary excretion of nitrosodimethylamine and nitrosoproline in humans: interindividual and intraindividual differences and the effect of administered ascorbic acid and α-tocopherol. Cancer Res., *46:* 5392–5400, 1986.
7. Cooper, S. W., Leymoyne, C., and Gaureau, D. Identification and quantitation of *N*-nitrosamines in human postmortem organs. J. Anal. Toxicol., *11:* 12–18, 1987.
8. Swann, P. F., Coe, A. M., and Mace, R. Ethanol and dimethylnitrosamine and diethylnitrosamine metabolism and disposition in the rat. Possible relevance to the influence of ethanol on human cancer incidence. Carcinogenesis (Lond.), *5:* 1337–1343, 1984.
9. Gricute, L., Castegnaro, M., and Berezeit J. C. Influence of ethyl alcohol on carcinogenesis with *N*-nitrosodimethylamine. Cancer Lett., *13:* 345–352, 1981.
10. Anderson, L. M., Harrington, G. W., Pylypiw, H. M., Hagiwara, A., and Magee, P. N. Tissue levels and biological effects of *N*-nitrosodimethylamine in mice during chronic low or high dose exposure with or without ethanol. Drug Metab. Dispos., *14:* 733–739, 1986.
11. Anderson, L. M. Increased numbers of *N*-nitrosodimethylamine-initiated lung tumors in mice by chronic co-administration of ethanol. Carcinogenesis (Lond.), *9:* 1717–1719, 1988.
12. Mico, B. A., Swagzdis, J. E., Hu, H. S., Keefer, L. K., Oldfield, N. F., and Garland, W. A. Low-dose *in vivo* pharmacokinetics and deuterium isotope effects of *N*-nitrosodimethylamine in rats. Cancer Res., *45:* 6280–6285, 1985.
13. Streeter, A. J., Nims, R. W., Wu, P. P., and Shefels, P. R. Comparative toxicokinetics of several nitrosamines in the rat and the hamster. Proc. Am. Assoc. Cancer Res., *30:* 151, 1989.
14. Swann, P. F. Metabolism of nitrosamines: observation on the effect of alcohol on nitrosamine metabolism and on human cancer. Banbury Rep., *12:* 53–68, 1982.
15. Gombar, C. T., Pylypiw, H. M., and Harrington, G. W. Pharmacokinetics of *N*-nitrosodimethylamine in beagles. Cancer Res., *47:* 343–347, 1987.
16. Gombar, C. T., Harrington, G. W., Pylypiw, H. M., Bevill, R. F., Thurmon, J. C., Nelson, D. R., and Magee, P. N. Pharmacokinetics of *N*-nitrosodimethylamine in swine. Carcinogenesis (Lond.), *9:* 1351–1354, 1988.
17. Dedrick, R. L. Animal scale-up. J. Pharmacokinet. Biopharm., *11:* 435–460, 1973.
18. Boxenbaum, H., and Ronfeld, R. Interspecies pharmacokinetic scaling and the Dedrick plots. Am. J. Physiol., *245:* R768–R774, 1983.
19. Boxenbaum, H. Interspecies scaling, allometry, physiological time, and the ground plan of pharmacokinetics. J. Pharmacokinet. Biopharm., *10:* 201–227, 1982.
20. Rowland, M. Physiological pharmacokinetic models and interanimal species scaling. Pharmacol. Ther., *29:* 49–68, 1985.
21. Mordenti, J. Pharmacokinetic scale-up: accurate prediction of human pharmacokinetic profiles from animal data. J. Pharm. Sci., *74:* 1097–1099, 1985.
22. Weiss, M., Sziegoleit, W., and Forester, W. Dependence of pharmacokinetic parameters on body weight. Int. J. Clin. Pharmacol., *15:* 572–575, 1977.
23. Boxenbaum, H. Interspecies variation in liver weight, hepatic blood flow, and antipyrine intrinsic clearance: extrapolation of data to benzodiazepines and phenytoin. J. Pharmacokinet. Biopharm., *8:* 165–176, 1985.
24. Bergerova-Fiserova, V., and Hughes, H. C. Species differences on bioavailability on inhaled vapors and gases. *In:* V. Fiserova-Bergerova, (ed.), Modeling of Inhalation Exposure to Vapors: Uptake, Distribution, and Elimination, Vol. 2, pp. 97–106. Boca Raton, FL: CRC Press, Inc., 1983.
25. Pylypiw, H. M., Zimmerman, F., Harrington, G. W., and Anderson, L. M. Apparatus for trace determination of volatile *N*-nitrosamines in small samples. Anal. Chem., *57:* 2996–2997, 1985.
26. Gomeni, R. PHARM—an interactive graphic program for individual and population pharmacokinetic parameter estimation. Comput. Biol. Med., *14:* 25–34, 1985.
27. Gibaldi, M., and Perrier, D. Pharmacokinetics, Ed. 2. New York: Marcel Dekker, Inc., 1982.
28. Skipper, P. L., Tomera, J. F., Wishnok, J. S., Brunengraber, H., and Tannenbaum, S. R. Pharmacokinetic model for *N*-nitrosodimethylamine based on Michaelis-Menten constants determined with the isolated perfused rat liver. Cancer Res., *43:* 4786–4790, 1983.
29. Streeter, A. J., Nims, R. W., Sheffels, P. R., Heur, Y.-H., Yang, C. S., Mico, B. A., Gombar, C., and Keefer, L. K. Metabolic denitrosation of *N*-nitrosodimethylamine *in vivo* in the rat. Cancer Res., *50:* 1144–1150, 1990.

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The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Interspecies Scaling of the Pharmacokinetics of *N*-Nitrosodimethylamine

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Cancer Res 1990;50:4366-4370.

Carcinogenesis vol.9 no.8 pp.1351–1354, 1988

Pharmacokinetics of *N*-nitrosodimethylamine in swine

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The pharmacokinetics of *N*-nitrosodimethylamine (NDMA) have been studied in swine. They were studied following i.v. administration of 0.1, 0.5 and 1.0 mg/kg, and following oral doses of 1.0 and 5.0 mg/kg of NDMA. Following a bolus i.v. dose, the concentration of NDMA in blood declined biphasically with a mean distribution half-life of 7 min and a mean elimination half-life of 28 min. The areas under the blood concentration versus time curves (AUC) were roughly proportional to dose indicating that the pharmacokinetics in this dose range were first order. The mean systemic clearance from blood was 65.8 ml/min/kg, the steady-state volume of distribution was 1.4 l/kg, and the mean residence time was 20 min. Following the oral doses, the AUC and peak concentration in blood were not proportional to the dose. It is likely that the pharmacokinetics at the lower dose were first order, but at the higher dose the pharmacokinetics were no longer first order because metabolism was saturated. The bioavailability of the 1.0 mg/kg dose was 67%. Since the clearance was probably due to metabolism and the clearance from blood exceeded hepatic blood flow, the high bioavailability suggests that extrahepatic metabolism plays an important role in the systemic clearance of NDMA in swine.

Introduction

N-Nitroso compounds comprise a large class of potent chemical carcinogens. One subclass, the *N*-nitrosamines, are indirect-acting carcinogens that require metabolic activation to exert their toxic effect. These compounds are particularly interesting because they display a striking organ specificity that appears to be related to the chemical structure of the nitrosamine and the organ distribution of the enzymes required for their activation (1). It has not yet been proven that *N*-nitrosamines cause any human cancer, but a link is very possible because they have been shown to be carcinogenic in a wide variety of species, they can be detected in the environment, and the potential exists for precursor amines to be nitrosated in the acid environment in the stomach (1–3).

In vivo formation of nitrosamines is a particularly difficult human health problem because it is impossible to prevent exposure of humans to precursor amines, nitrate and nitrite are normal constitutents of saliva, and the pH of the stomach is conducive to nitrosation reactions. It has been suggested that formation of small amounts of nitrosamines in the stomach may not pose a significant threat to human health because the liver would clear almost all of the nitrosamine in a single pass (4). *N*-Nitrosodimethylamine (NDMA*) is extracted efficiently by the liver in rats; the oral bioavailability of NDMA in rats is only 8% (i.e. >90% of the NDMA is metabolized in a single pass) (5). However, the bioavailability of NDMA in dogs has been shown to be >90% (6).

Swine provide an attractive model for physiologic and pathophysiologic studies because their body size, dietary habits, digestive physiology and other physiologic processes are similar to humans (7). The pig is an omnivore that will eat almost any feed presented to it. This makes the pig a particularly good model for studying *in vivo* nitrosation. Preliminary studies have demonstrated the utility of the pig model for pharmacokinetic and metabolism studies of nitrosamines (8), and the present study provides detailed data on the pharmacokinetics of NDMA in swine.

The two main objectives of the present study were to determine the basic pharmacokinetic parameters for NDMA in swine as part of a program to investigate interspecies scaling of NDMA pharmacokinetics, and to provide data on the oral bioavailability of NDMA to assess the feasibility of *in vivo* nitrosation studies and to aid in the interpretation of nitrosation data.

Materials and methods

Chemicals

NDMA was purchased from Sigma Chemical Co. (St Louis, MO). *N*-Nitroso-di[methyl-^{14}C]amine was synthesized from di[methyl-^{14}C]amine by the method of Dutton and Heath (9). Morpholine (Aldrich Chemical Co., Milwaukee, WI) was double distilled, and the second distillate was collected and stored under nitrogen gas. Antifoam B was purchased from Fisher Scientific Co. (King of Prussia, PA). All other chemicals and solvents were ACS reagent grade or better.

Animals and treatment

Crossbred pigs weighing 38–42 kg were obtained from a specific pathogen-free herd of Chester White × Duroc pigs, maintained by the University of Illinois College of Veterinary Medicine. Animals were maintained in enclosed communal pens and fed a balanced corn–soya feed. The experimental animals were prepared with halothane anesthesia for surgical implantation of a femoral cannula for blood sampling. After surgery the animals were placed in individual cages to recover for 48 h before dosing. Oral doses were administered to animals that had been fasted for 24 h. The oral doses were administered via a stomach tube installed with the aid of a mouth speculum. The tube was flushed with sterile water after dosing. Intravenous doses were administered in saline via a femoral vein cannula which was flushed with heparinized saline after dosing. Samples of blood were collected from a femoral vein cannula with a syringe at various times after dosing, placed in heparinized plastic bottles which contained several crystals of ascorbic acid, and frozen in liquid nitrogen.

Determination of NDMA in blood

The concentration of NDMA in blood was determined by gas chromatography–thermal energy analyzer (CG–TEA) as described by Pylypiw *et al.* (10). Briefly, 2.0 ml of blood was simultaneously distilled and extracted into methylene chloride using a specially designed distillation–extraction apparatus. Following

Abbreviations: NDMA, *N*-nitrosodimethylamine; Cl_s, systemic clearance; V_{ss}, steady-state volume of distribution; MRT, mean residence time; AUC, area under the blood concentration–time curve from zero to infinity; MAT, mean absorption time; $T_{1/2}$, apparent elimination half-life; F, bioavailability; Cl_{po}, oral clearance; Cl_{int}, intrinsic clearance; f_B, free fraction in blood; $T1/2\alpha$, distribution half-life; $T1/2\beta$, elimination half-life; C_{max}, maximum blood concentration; T_{max}, time to reach C_{max}.

overnight distillation, the methylene chloride extract was concentrated and the NDMA concentration was determined by GC–TEA using a thermal energy analyzer, Model TEA-502 (Thermo-Electron Corp, Waltham, MA), interfaced with a Hewlett Packard Model 5791A packed-column gas chromatograph (Avondale, PA).

Plasma protein binding

The binding of [^{14}C]NDMA to plasma protein was measured in heparinized pig plasma over an initial concentration range of 1–1000 ng/ml using the Centrifree micropartition system (Amicon Corp, Danvers, MA). Preliminary experiments demonstrated that [^{14}C]NDMA did not adhere to the filtration device.

Pharmacokinetic calculations

The blood concentration versus time data following i.v. administration were fitted to a two-compartment open model using the PHARM program of Gomeni (11). Fitting was done either by curve peeling of the log-transformed data, or by weighted ($1/y$) non-linear regression. The goodness of the fit was assessed by visual inspection of the fit and the residual plot. Noncompartmental methods were used to calculate systemic clearance (Cl_s), steady-state volume of distribution (V_{ss}) and mean residence time (MRT) (12). The area under the blood concentration versus time curve from time zero to infinity (AUC) was calculated to the last data point using the linear trapezoidal rule, and the area from the last data point to infinity was estimated by dividing the concentration at the last time point by the apparent elimination rate constant.

The absorption half-life was calculated using equation 1 where MAT, the mean absorption time, is the difference between the MRT after oral administration and the MRT after i.v. administration ($T_{1/2}$ is the apparent elimination half-life).

$$\text{Absorption } T_{1/2} = 0.693*\text{MAT} \quad (1)$$

The apparent oral bioavailability (F) was determined using equation 2.

$$F = \frac{\text{AUC}_{po} * \text{Dose}_{i.v}}{\text{AUC}_{i\,v.} * \text{Dose}_{po}} \quad (2)$$

Oral clearance (Cl_{po}) is the dose divided by AUC_{po}. If the assumption is made that the dose is completely absorbed and that elimination is due only to hepatic metabolism, then the intrinsic hepatic clearance (Cl_{int}) is related to the oral clearance by equation 3 where f_B is the free fraction in blood.

$$Cl_{po} = f_B * Cl_{int} \quad (3)$$

Results

Pharmacokinetic parameters were determined following i.v. administration of NDMA to swine at doses of 0.1, 0.5 and 1.0 mg/kg (Table I). Following bolus i.v. administration of each dose, the concentration of NDMA in blood declined in a biphasic manner (Figure 1) with a distribution half-life ($T1/2\alpha$) of 7 min, and a mean elimination half-life ($T1/2\beta$) of 28 min. At the lowest dose the concentration of NDMA in blood reached the sensitivity limit of the assay before a good characterization of the elimination phase could be made. The pharmacokinetics of NDMA over this dose range appear to be linear (i.e. first order) since the AUC was roughly proportional to the dose. The mean systemic clearance of NDMA from blood for all animals was 65.8 ml/min/kg, and the steady-state volume of distribution was 1.4 l/kg. The mean residence time, a 'half-life like' parameter not subject to the difficulties of modelling, averaged 20 min.

Over an initial concentration range of 1–1000 ng/ml, NDMA did not bind to plasma proteins *in vitro*.

NDMA appeared to be rapidly absorbed following oral administration of 1.0 and 5.0 mg/kg with the concentration in blood reaching a peak ~23 min after dosing (Figure 2, Table II). The pharmacokinetics of NDMA were clearly non-linear at the 5.0 mg/kg dose because the AUC and the maximum blood concentration (C_{max}) were not proportional to the dose (Table II). The pharmacokinetics at the lower dose were probably linear because the concentrations in blood following administration of the higher dose were substantially greater than those achieved after any of the i.v. doses, whereas the lower oral dose resulted in concentrations less than those observed following the 0.5 mg/kg i.v. dose. The non-linearity, which is probably due to saturation of metabolism, invalidates the calculation of Cl_{po}, MRT and F for the higher oral dose. The terminal half-life after oral dosing (~60 min) was longer than the elimination half-life observed after i.v. dosing suggesting that the terminal half-life is an absorption rather than an elimination half-life. However, the noncompartmental parameter MRT can be used to estimate the absorption half-life without having to fit the data to a model. Using the mean MRT value for the low dose oral and i.v. data and equation 1, the absorption half-life was estimated to be 34 min. It is not clear, then, whether the terminal half-life is absorption limited or not. The oral bioavailability of NDMA in swine was estimated to be 67%.

Table I. Pharmacokinetic parameters for NDMA administered i.v. to swine at three different doses

Pig no.	AUC (min*ng/ml)	Cl_s (ml/min/kg)	V_{ss} (l/kg)	MRT (min)	$T1/2\alpha$ (min)	$T1/2\beta$ (min)
0.1 mg/kg						
1	1107	90.3	1.5	16	9	30
2	1945	51.4	0.8	15	1	15
Mean	1526	70.9	1.2	16	5	23
0.5 mg/kg						
3	6718	74.4	1.5	20	5	28
4	7201	69.4	1.8	25	8	34
5	6546	76.4	2.3	30	9	38
Mean	6822	73.4	1.9	25	7	33
SD	340	3.6	0.4	5	2	5
1.0 mg/kg						
6	20 904	47.8	1.0	21	5	25
7	18 940	52.8	1.2	23	14	30
8	15 614	64.0	0.7	12	3	21
Mean	18 486	54.9	1.0	19	7	25
SD	2674	8.3	0.3	6	6	5
Overall mean		65.8	1.4	20	7	28
SD		14.7	0.5	6	4	7

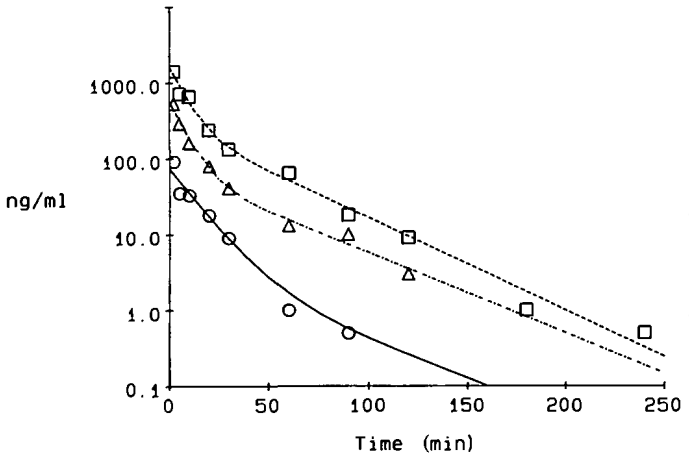

Fig. 1. Typical blood concentration versus time profiles for swine given 0.1 mg/kg (○—○), 0.5 mg/kg (△—△) and 1.0 mg/kg (□—□) NDMA i.v. The lines represent the fitted curve for each set of data.

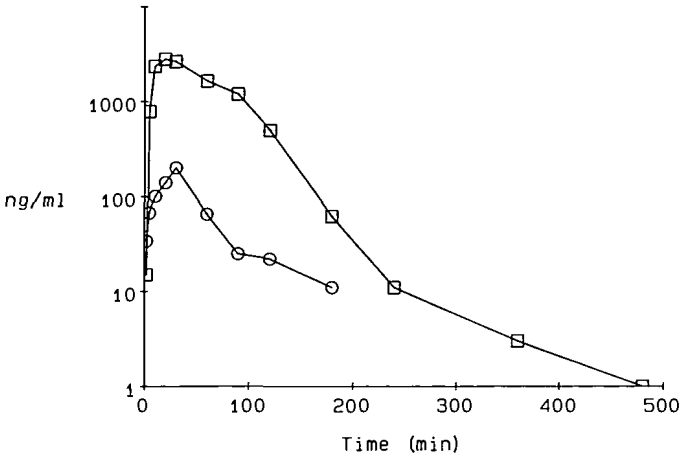

Fig. 2. Typical blood concentration versus time profile for swine given 1.0 mg/kg (○—○) and 5.0 mg/kg (□—□) NDMA orally.

Table II. Pharmacokinetic parameters for NDMA administered orally to swine at two different doses

Pig no.	AUC (min*ng/ml)	C_{max} (ng/ml)	T_{max} (min)	$T_{1/2}$ (min)	Cl_{po} (ml/min/kg)	MRT (min)	F^a (%)
1.0 mg/kg							
9	7855	130	10	42	127	65	58
10	11 344	200	30	51	88	64	83
11	8011	101	30	58	125	78	59
Mean	9070	144	23	50	113	69	67
SD	1971	51	12	8	22	8	14
5.0 mg/kg							
12	172 698	2300	30	74			
13	215 128	2800	20	69			
14	177 321	1550	20	59			
Mean	188 382	2217	23	67			
SD	23 277	629	6	8			

[a]Bioavailability calculated relative to mean AUC for 0.5 mg/kg i.v. dose.

Discussion

Extrapolation of carcinogenicity data from laboratory animals to humans is a difficult task because chemical carcinogenesis is a multi-step process involving many factors. Metabolic activation, DNA repair processes, inherent tissue susceptibility to the carcinogen and other processes may all play important roles in chemical carcinogenesis. The pharmacokinetics of a compound, its absorption, distribution, metabolism and excretion characteristics, clearly play an important role because these factors determine the exposure of tissues to the compound and metabolism is often involved in the activation of many carcinogens.

Pharmacokinetics can be a useful tool in chemical carcinogenesis research for several reasons. (i) If the pharmacokinetics have been studied in several species, the pharmacokinetic parameters may vary predictably between species such that they can be scaled, or physiologic pharmacokinetic models may be employed to perform interspecies scaling as has been accomplished with several therapeutic agents (13–15). The ability to scale the pharmacokinetics helps to put carcinogenicity data, based on dose, into perspective. (ii) An understanding of the pharmacokinetics gives an appreciation of the overall fate of the molecule *in vivo* (e.g. the relative amount of renal versus metabolic clearance). (iii) Comparison of blood concentration versus time curves for the carcinogen given by different routes of administration can give insight into which organs are involved in the clearance of the compound. Ideally, this should be followed by investigations utilizing specialized techniques such as isolated perfused organs to determine precisely the role that certain organs play in clearance. (iv) When administration of the compound is by several routes, including the putative route of exposure (oral, percutaneous, inhalation, etc.), the pharmacokinetic data can be used to estimate the systemic exposure to the carcinogen from these routes of administration.

There are limitations to the usefulness of pharmacokinetic data in carcinogenesis that should be appreciated as well. First, in the case of a chemical carcinogen requiring metabolic activation the situation is complicated because the clearance may involve non-metabolic processes (i.e. renal or biliary clearance) and metabolic clearance. The metabolic clearance will encompass detoxifying reactions and those that activate the compound to the ultimate carcinogen. Pharmacokinetics is the study of the rates at which these processes can occur, but if we are concerned with the metabolic activation of a carcinogen a distinction must be made between the rate and extent of reaction. For instance, if 50% of the dose of a given compound is metabolized by a route generating the ultimate carcinogen, the rate of that reaction may vary considerably between two species, but in both species the same fraction of the dose may be metabolized by that pathway and the same amount of ultimate carcinogen will be produced. The same concept applies when studying high and low doses of a carcinogen within a species. At high doses the enzyme producing the activated carcinogen may be saturated, i.e. the rate of reaction is no longer proportional to concentration of substrate, or, stated another way, the clearance is not constant. If that occurs, but no other clearance mechanism is available for removal of the compound, then the same fraction of the dose will be metabolized by that pathway. The rate of removal will be different, but the extent of reaction will be the same.

Second, the usefulness of pharmacokinetic data is limited because major routes of metabolism, and hence the bulk of the clearance, may involve detoxification pathways that have little relevance to the carcinogenic effect. Minor metabolites may be responsible for the toxic effect, and the pharmacokinetic data may not have the 'resolution' to determine critical differences between species.

The present study was undertaken as part of a program to investigate the pharmacokinetics of NDMA in a variety of species to allow eventual interspecies scaling of the pharmacokinetics. As noted in the Introduction, swine have many physiological similarities with humans, and for the purposes of interspecies scaling they are attractive because their body weight is similar to humans.

The basic pharmacokinetic parameters, clearance and volume of distribution, were obtained by i.v. administration of NDMA to swine. The systemic clearance of NDMA from blood was 65.8 ml/min/kg. The relative contribution of renal and non-renal clearance to the total systemic clearance was not determined in this study, but renal clearance of NDMA has been shown to be negligible in rats and dogs (5,6). It is reasonable to assume that renal clearance of NDMA is low in pigs as well. The steady-state volume of distribution of NDMA was 1.4 l/kg. Since NDMA does not bind to plasma protein, and the blood/plasma concentration ratio is essentially 1.0, this volume term is consistent with wide distribution throughout the body with no substantial concentration in tissues.

The oral data are interesting because they demonstrate that the metabolism of NDMA is saturated at a dose of 5 mg/kg, and that, even at a dose where metabolism is apparently not saturated, the bioavailability is 67%. That means that 67% of an orally administered dose of NDMA passes through the liver into the systemic circulation. This situation is similar to that observed in dogs, but very different from the situation in rats. At low oral doses in rats systemic exposure is low, and this has been used as an explanation for the observation that low oral doses of NDMA result primarily in liver tumors. Since a large portion of an oral dose of NDMA in swine and dogs (6) reaches the systemic circulation, it raises questions regarding the target organ of low doses in these species. Unfortunately, the carcinogenicity of NDMA has not been tested in either species.

The pharmacokinetic data, taken together, give some insight into the organs involved in the clearance of NDMA in swine. If it is assumed that the clearance of NDMA is due to metabolism, then the liver cannot be the sole clearing organ. The maximum clearance of NDMA from blood by the liver is the blood flow to the liver, which in swine is ~44 ml/min/kg (14). Since the systemic clearance of NDMA in swine is greater than hepatic blood flow, then organs other than the liver must be involved in the clearance of NDMA. Also, if the hepatic extraction ratio (i.e. the ratio of hepatic clearance to hepatic blood flow) was high, then the oral bioavailability of NDMA would be low. Since the mean bioavailability was 67% this suggests that the liver is not the only organ that contributes to the systemic clearance of NDMA in swine.

A similar situation (i.e. high bioavailability and clearance exceeding hepatic blood flow) was observed in dogs (6), and an explanation put forward was that clearance by the lungs may play an important role in the clearance of NDMA. The lungs should be capable of metabolizing NDMA, and a fraction of the dose may be eliminated in the expired air. Even if the pulmonary extraction ratio is modest, the clearance may be significant because the blood flow to the lungs is equal to cardiac output. In the experimental determination of bioavailability, the AUC following oral and i.v. administration are compared. The asumption is that the entire dose reaches the systemic circulation upon i.v. administration. If there is a significant pulmonary first-pass clearance, then the assumption is violated and the AUC after i.v. administration is erroneously low. This will artificially inflate the bioavailability estimate (17). Clear determination of hepatic and pulmonary clearance is needed to understand completely the pharmacokinetics of NDMA in these species.

The present study, along with data from rats (5), dogs (6) and rabbits (16) already published, and studies in progress in primates and mice, will allow interspecies scaling of the pharmacokinetics of NDMA to be performed. This study also provides the foundation for studies utilizing swine as a model for *in vivo* nitrosation and studies designed to investigate the pharmacokinetics of NDMA in more detail.

Acknowledgement

This work was supported in part by NIH Grant CA 43342-01.

References

1. Preussmann,R. and Stewart,B.W. (1984) *N*-Nitroso carcinogens. In Searle,C.E. (ed) *Chemical Carcinogens* (2nd edn). *Am. Chem. Soc. Monogr.*, **182**, 643–828.
2. Mirvish,S.S. (1975) Formation of *N*-nitroso compounds: chemistry, kinetics and *in vivo* occurrence. *Toxicol. Appl. Pharmacol.*, **31**, 325–351.
3. Magee,P.N. (ed.) (1982) *Nitrosamines and Human Cancer*. Banbury Report No. 12. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
4. Diaz Gomez,M.I., Swann,P.F. and Magee,P.N. (1977) The absorption and metabolism in rats of small oral doses of dimethylnitrosamine: implication for the possible hazard of dimethylnitrosamine in human food. *Biochem. J.*, **164**, 497–500.
5. Mico,B., Swagzdis,J.E., Hu,H.S.-W., Keefer,L.K., Oldfield,N.F. and Garland,W.A. (1985) Low dose *in vivo* pharmacokinetics and deuterium isotope effect studies of *N*-nitrosodimethylamine in rats. *Cancer Res.*, **45**, 6280–6285.
6. Gombar,C.T., Pylypiw,H.M. and Harrington,G.W. (1987) Pharmacokinetics of *N*-nitrosodimethylamine in beagles. *Cancer Res.*, **47**, 343–347.
7. Tumbleson,M.E. (ed.) (1985) *Swine in Biomedical Research*, Vols. I–III. Plenum Press, New York.
8. Harrington,G.W., Magee,P.N., Pylypiw,H.M., Bevill,R.F., Nelson,D.R. and Thurmon,J.C. (1987) The pig as an animal model for the study of nitrosamine metabolism. In *N-Nitroso Compounds—Relevance to Human Cancer*. Proceedings of the Ninth International Symposium on *N*-Nitroso Compounds, IARC Publication, Lyon, no. 84, p. 132.
9. Dutton,A.H. and Heath,D.F. (1956) The preparation of [^{14}C]dimethylamine and [^{14}C]dimethylnitrosamine. *J. Chem. Soc.*, 1892.
10. Pylypiw,H.M., Zimmerman,F. and Harrington,G.W. (1985) Apparatus for trace determination of volatile *N*-nitrosamines in small samples. *Anal. Chem.*, **57**, 2996–2997.
11. Gomeni,R. (1984) PHARM—an interactive graphic program for individual and population pharmacokinetic parameter estimation. *Comput. Biol. Med.*, **14**, 25–34.
12. Gibaldi,M. and Perrier,D. (1982) *Pharmacokinetics*, 2nd edn. Marcel Dekker, Inc., New York.
13. Dedrick,R. (1973) Animal scale-up. *J. Pharmacokinet. Biopharm.*, **1**, 435–460.
14. Boxenhaum,H. (1980) Interspecies variation in liver weight, hepatic blood flow, and antipyrine intrinsic clearance: extrapolation of data to benzodiazepines and phenytoin. *J. Pharmacokinet. Biopharm.*, **8**, 165–176.
15. Mordenti,J. (1985) Pharmacokinetic scale-up: accurate prediction of human pharmacokinetic profiles from animal data. *J. Pharm. Sci.*, **74**, 1097–1099.
16. Swann,P.F. (1982) *Metabolism of Nitrosamines: Observations on the Effect of Alcohol on Nitrosamine Metabolism and Human Cancer*. Banbury Report No. 12. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 53–68.
17. Chiou,W.L. (1979) Potential pitfalls in the conventional pharmacokinetic studies: effects of the initial mixing of drug in blood and the pulmonary first-pass elimination. *J. Pharmacokinet. Biopharm.*, **7**, 527–536.

Received on September 10, 1987; revised on April 18, 1988; accepted on April 28, 1988

[CANCER RESEARCH 47, 343–347, January 15, 1987]

Pharmacokinetics of *N*-Nitrosodimethylamine in Beagles[1]

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ABSTRACT

The pharmacokinetics of *N*-nitrosodimethylamine (NDMA) has been studied in beagles. Four male beagles were given 0.5- and 1.0-mg/kg doses of NDMA i.v. and 1.0- and 5.0-mg/kg doses p.o., and at appropriate times after dosing blood samples were drawn and the concentration of NDMA was measured. The experiments were separated by at least 1 week. Following a bolus i.v. dose, the concentration of NDMA in blood declined biphasically with a mean distribution half-life of 19 min and a mean elimination half-life of 73 min. The areas under the blood concentration *versus* time curves were proportional to the dose indicating that the pharmacokinetics in this dose range were first order. The mean systemic clearance was 43.3 ml/min/kg, the volume of distribution at steady state was 1.9 liters/kg and the mean residence time was 45 min. The clearance of NDMA in the dog was entirely metabolic because no NDMA could be detected in urine after i.v. dosing. The areas under the curve and maximum concentration in blood after the two p.o. doses were not proportional to dose. The evidence suggests that the pharmacokinetics of the 1.0-mg/kg dose were first order, but at the 5.0-mg/kg dose the metabolism of NDMA was saturated. The bioavailability of the lower p.o. dose (*i.e.*, the fraction of the dose that reached the systemic circulation) averaged 93%. The high bioavailability was unexpected since, in the rat, the bioavailability of NDMA is only about 10%, and the systemic clearance in the dog exceeds hepatic blood flow. These data suggest that a substantial fraction of the systemic clearance is extrahepatic and that the pharmacokinetics of NDMA in higher species may be quite different from that observed in rodents.

INTRODUCTION

The carcinogenicity of NDMA[3] and related nitrosamines has been demonstrated in at least 39 species (1, 2), but there is no direct evidence linking nitrosamines to human cancer. The mechanism of the carcinogenicity of NDMA is generally believed to involve alkylation of DNA, and the alkylating moiety results from metabolic activation of NDMA. Indirect evidence, such as comparative *in vitro* metabolism using tissue slices (3), homogenates (4), or explant cultures (5–10), suggests that the metabolic pathway responsible for generating the ultimate carcinogen is operative in humans. Furthermore, DNA from humans poisoned with NDMA was shown to contain the methylated bases 7-methylguanine and O^6-methylguanine (11).

Extrapolation of carcinogenicity data from animals to humans is fraught with difficulty. The inherent susceptibility of tissues to the carcinogenic action of NDMA, the efficiency and fidelity of repair processes, quantitative and qualitative metabolic aspects, and the pharmacokinetics of the compound may be very different in humans. Some of these problems can be studied in isolation. For instance, the availability of a suitable data base may allow extrapolation of the pharmacokinetic data from animals to humans. Currently, the only species for which good pharmacokinetic data exist are the rat (12) and the rabbit (13). The pharmacokinetics of NDMA and deuterated NDMA following low doses to the rat was studied by Mico *et al.* (12). Other information on the pharmacokinetics in rats comes from a study by Skipper *et al.* who developed a pharmacokinetic model for NDMA based on *in vitro* data (14) and other studies in which pharmacokinetic information was obtained at very high doses, or indirectly through measurement of exhaled $^{14}CO_2$ or DNA alkylation after administration of radiolabeled NDMA (15–20).

These studies have shown that NDMA, when administered p.o. at low doses, is well absorbed from the gastrointestinal tract, but only a very small fraction, about 10%, of the dose passes through the liver into the general circulation. This was shown directly by measuring the concentration of NDMA in blood after p.o. or i.v. administration (12) and indirectly by measuring the extent of DNA alkylation in kidney relative to liver after p.o. and i.v. dosing (18–20). The extent of first pass metabolism in species other than the rat is not known.

Interspecies scaling of pharmacokinetic data is difficult, especially when the compound is cleared primarily by metabolism, and extrapolation is virtually impossible when data are available for only one or two species. If several species are studied, allometric analysis may allow a reasonable interspecies extrapolation (21–27).

The purpose of the present study, and others in progress, is to collect detailed information on the pharmacokinetics of NDMA in mammalian species other than the rat to eventually allow interspecies comparisons to be made and, if possible, to extrapolate the data to describe the pharmacokinetics of NDMA in humans.

MATERIALS AND METHODS

Chemicals. *N*-Nitrosodimethylamine was purchased from Sigma Chemical Co. (St. Louis, MO). *N*-Nitrosodi[*methyl*-^{14}C]amine was synthesized from di[*methyl*-^{14}C]amine, purchased from Amersham Corp. (Arlington Heights, IL), by the method of Dutton and Heath (28). Morpholine (Aldrich Chemical Co., Milwaukee, WI) was double distilled, and the second distillate was collected and stored under nitrogen gas. Antifoam B was purchased from Fisher Scientific Co. (King of Prussia, PA). All other chemicals and solvents were ACS reagent grade or better.

Animals and Treatments. Four male purebred beagles obtained from Marshall Farms USA, Inc. (North Rose, NY), were used in this study. The dogs were placed in free standing slings and catheters were placed in the right and left saphenous veins. For i.v. dosing, NDMA dissolved in 0.9% NaCl solution was administered as a bolus dose in a volume of 0.2 ml/kg in one leg, and at appropriate times after dosing 5-ml blood samples were drawn into heparinized syringes from the catheter in the contralateral leg. Blood samples were immediately frozen in an acetone-dry ice bath and stored at −80°C until analyzed. Dosing experiments p.o. were performed in a similar manner except that the NDMA, dissolved in water, was administered in gelatin capsules. After blood sampling was completed, the dogs were housed in stainless steel metabolism cages and urine was collected for 24 h. Immediately prior to dosing and 24 h after dosing, serum samples were obtained for analysis

Received 7/29/86; revised 10/1/86; accepted 10/8/86.

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[1] This work was supported in part by NIH Grant CA18618.

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[3] The abbreviations used are: NDMA, *N*-nitrosodimethylamine; AUC, area under the blood concentration *versus* time curve from time zero to infinity; Cl_s, systemic clearance from blood; $Cl_{p.o.}$, oral clearance; Cl_{int}, intrinsic hepatic clearance; V_{ss}, volume of distribution at steady state; MRT, mean residence time; C_{max}, maximum concentration in blood; t_{max}, time to maximum concentration; $t_{1/2}$, apparent elimination half-life following oral administration; $t_{1/2}\alpha$, half-life of the first disposition phase following i.v. administration; $t_{1/2}\beta$, half-life of the second disposition phase following i.v. administration.

of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and γ-glutamyl transferase activities, total protein concentration, and the albumin/globulin ratio.

Determination of NDMA in Blood and Urine. The concentration of NDMA in blood and urine was determined as described by Pylypiw *et al.* (29). Briefly, 2.0 ml of blood or 5.0 ml of urine were simultaneously distilled and extracted into methylene chloride using a specially designed distillation-extraction apparatus. Following concentration of the methylene chloride extract, NDMA was determined by gas chromatography-thermal energy analysis using a thermal energy analyzer, Model TEA-502 (Thermo-Electron Corp., Waltham, MA), interfaced with a Hewlett Packard Model 5791A packed column gas chromatograph (Avondale, PA).

Plasma Protein Binding. The binding of [^{14}C]NDMA to plasma protein was measured in fresh heparinized dog plasma over an initial concentration range of 1 to 1000 ng/ml using the Centrifree micropartition system (Amicon Corp., Danvers, MA). Preliminary experiments demonstrated that [^{14}C]NDMA did not adhere to the filtration device.

Pharmacokinetic Calculations. The blood concentration *versus* time data following i.v. administration were fit to a two compartment open model using the PHARM program of Gomeni (30). Fitting was done either by curve peeling of the log transformed data or by weighted ($1/y$) nonlinear regression. The goodness of the fit was assessed by visual inspection of the fit and the residual plot. Noncompartmental methods were used to calculate Cl_s, V_{ss}, and MRT (31). The area under the blood concentration *versus* time curve from time zero to the last data point was calculated using the trapezoidal rule. The area from the last data point to infinity was estimated by dividing the concentration at the last time point by the apparent elimination rate constant.

The absorption half-life was calculated using Equation A where MAT, the mean absorption time, is the difference between the MRT after p.o. administration and the MRT after i.v. administration.

$$\text{Absorption } t_{½} = 0.693 * \text{MAT} \quad \text{(A)}$$

The apparent oral bioavailability (F) was determined using Equation B.

$$F = \frac{\text{AUC}_{\text{p.o.}} * \text{dose}_{\text{i.v.}}}{\text{AUC}_{\text{i.v.}} * \text{dose}_{\text{p.o.}}} \quad \text{(B)}$$

$Cl_{\text{p.o.}}$ is the dose divided by $\text{AUC}_{\text{p.o.}}$. If we assume that the dose is completely absorbed and that elimination is due only to hepatic metabolism, then the intrinsic hepatic clearance (Cl_{int}) is related to the oral clearance by Equation C where f_B is the free fraction in blood.

$$Cl_{\text{p.o.}} = f_B * Cl_{\text{int}} \quad \text{(C)}$$

RESULTS

The concentration of NDMA in whole blood following an i.v. bolus dose decreased biphasically (Fig. 1). The half-life of the distributive phase was 18 ± 4 min in dogs given 0.5 mg/kg, and the elimination half-life was 72 ± 8 min. The values from dogs given 1.0 mg/kg were very similar (Table 1). The AUCs for the two doses used were roughly proportional to the dose indicating that the pharmacokinetics of NDMA was linear (first order) in this dose range. The systemic clearance from blood ranged from 33.9 to 52.9 ml/min/kg. All of these clearance values approach or exceed hepatic blood flow in the dog (~40 ml/min/kg) (32) suggesting that the liver is not the only organ involved in the clearance of NDMA. The volume of distribution at steady state was about 2.0 liters/kg. The mean residence time, which represents the time needed for 63.2% of the dose to be eliminated, is a "half-life-like" term that is not susceptible to the vagaries of curve fitting. The values for the mean residence time in these dogs showed remarkably low variability (Table 1).

The dogs given the 0.5-mg/kg dose were housed in metabolism cages and urine was collected for 24 h. No NDMA was detected in the urine suggesting that the systemic clearance of NDMA was totally metabolic and that the renal clearance of NDMA was essentially zero. Since NDMA may have been reabsorbed from the urinary bladder, the true renal clearance may be greater than zero. Practically, however, the renal clearance is negligible.

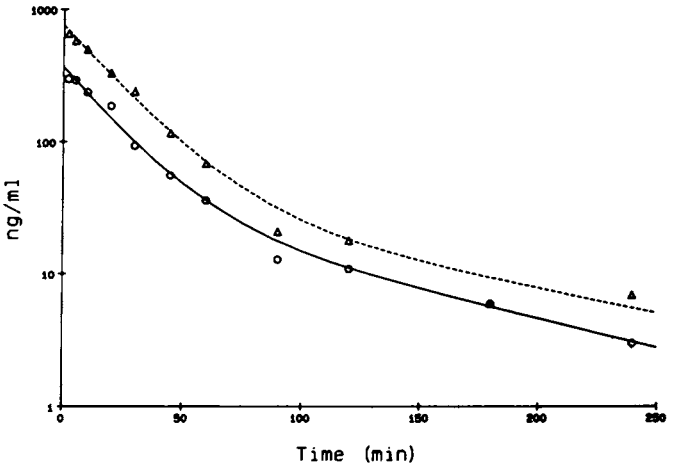

Fig. 1. Blood concentration *versus* time profile for dog 3, given 0.5 mg/kg (○) and 1.0 mg/kg (△) NDMA i.v.

Table 1 *Pharmacokinetic parameters for N-nitrosodimethylamine administered i.v. to beagles*

Dog	AUC (min•ng/ml)	Cl_s (ml/min/kg)	V_{ss} (liters/kg)	MRT (min)	$t_{½}\alpha$ (min)	$t_{½}\beta$ (min)
			0.5 mg/kg			
1	9,284	53.9	2.3	43	17	61
2	10,508	47.6	2.1	45	22	75
3	10,370	48.2	2.2	45	14	70
4	13,362	37.4	1.7	45	20	81
Mean	10,881	46.8	2.1	45	18	72
SD	1,742	6.9	0.3	1	4	9
			1.0 mg/kg			
1	26,366	37.9	1.8	47	25	41
2	25,561	39.1	1.4	37	17	49
3	20,843	48.0	2.2	45	15	81
4	29,461	33.9	1.5	45	19	124
Mean	25,558	39.7	1.7	44	19	74
SD	3,565	5.9	0.3	4	4	38

The pharmacokinetics following 1.0- and 5.0-mg/kg doses administered p.o. were studied as well. A typical blood concentration-time curve (Fig. 2), and the individual pharmacokinetic parameters (Table 2) illustrate some important points. Unlike the dose range used i.v., the pharmacokinetics of the 5.0-mg/kg p.o. dose was clearly nonlinear. The blood concentration-time curves did not decline in parallel, and the curve for the higher dose showed signs of saturated metabolism (Fig. 2). Consistent with this were the observations that the AUC and C_{max} values were not proportional to dose. The pharmacokinetics of the 1.0-mg/kg dose was probably first order since that same dose administered i.v. was first order, and the concentrations of NDMA in blood following 1.0 mg/kg p.o. did not exceed those observed after i.v. dosing. Therefore, the pharmacokinetic parameters calculated for the 1.0-mg/kg dose are valid.

The oral clearance can be considered to be a good approximation of the intrinsic hepatic clearance if certain assumptions are made. These assumptions are that the dose is totally ab-

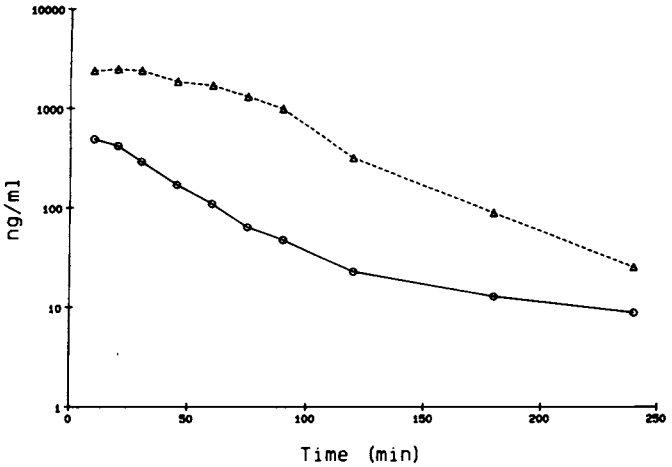

Fig. 2. Blood concentration *versus* time profile for dog 3, given 1.0 mg/kg (○) and 5.0 mg/kg (△) NDMA p.o.

Table 2 *Pharmacokinetic parameters for N-nitrosodimethylamine administered p.o. to beagles*

Dog	AUC (min•ng/ml)	C_{max} (ng/ml)	t_{max} (min)	$t_{½}$ (min)	$Cl_{p.o.}$ (ml/min/kg)	MRT (min)	F^a (%)
			1.0 mg/kg				
1	15,176	193	30	43	65.9	70	58
2	29,934	501	20	46	33.4	56	117
3	21,924	488	10	66	45.6	57	105
4	27,065	512	20	51	36.9	61	92
Mean	23,525	424	20	52	45.5	61	93
SD	6,478	154	8	10	14.6	6	26
			5.0 mg/kg				
1	224,183	2,434	20	34	22.3	67	170
2	236,686	2,896	30	36	21.1	68	185
3	196,678	2,455	20	40	25.4	58	189
4	221,768	2,923	30	45	22.5	65	151
Mean	219,829	2,677	25	39	22.8	65	174
SD	16,761	269	6	5	1.8	5	17

[a] Bioavailability calculated relative to the 1.0-mg/kg i.v. dose.

sorbed, that the free fraction of the compound in blood is 1, and that the liver is the only site of metabolism of the compound. Since the bioavailability is close to 100%, it is apparent that NDMA is completely absorbed from the gastrointestinal tract. The plasma protein binding of [^{14}C]NDMA, measured over an initial concentration range of 1 to 1000 ng/ml, was zero, and the blood/plasma concentration ratio of [^{14}C]NDMA was approximately 1 (data not shown). Therefore, it is reasonable to assume that the free fraction in blood equals 1. The $Cl_{p.o.}$ of NDMA in dogs is approximately 45 ml/min/kg.

The absorption half-life of NDMA can be estimated from the difference between the p.o. and i.v. mean residence times (31). Using this method, the absorption half-life for NDMA was 12 min.

The bioavailability of NDMA, which is the fraction of a p.o. dose that reaches the systemic circulation, was remarkably high and variable in the dogs ranging from 58 to 117%.

DISCUSSION

In the present study the pharmacokinetics of NDMA in dogs has been studied, and the most striking result is the observation that, in spite of a high systemic clearance, the oral bioavailability of NDMA is high. There are several explanations for this anomalous result that can be considered. (*a*) NDMA may be unstable in blood. This can be ruled out because NDMA added to blood *in vitro* and in actual biological samples incubated at 37°C was found to be very stable (data not shown). (*b*) At the doses used the first pass metabolism of NDMA was saturated. This is certainly true for the 5.0-mg/kg dose, but the 1.0-mg/kg dose appears to follow first order kinetics, the pharmacokinetics of 1.0 mg/kg administered i.v. are first order, and the concentrations of NDMA in blood following p.o. administration of 1.0 mg/kg were not higher than after the same dose i.v. (*c*) Since this was a crossover study and the dogs were given the i.v. doses first, significant liver damage occurred which decreased the hepatic clearance of the subsequent p.o. doses. This does not appear to be the case because activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ-glutamyl transferase in serum, and total protein concentration were within normal limits throughout the experiment except for a mild elevation of alanine aminotransferase and alkaline phosphatase in dog 3 after the first i.v. dose and before the second i.v. dose. (*d*) The most likely explanation is that a substantial fraction of the i.v. dose was metabolized in a single pass through the lung resulting in a pulmonary first pass effect. This would tend to lower the AUC after i.v. dosing and inflate the bioavailability values. It must be remembered that the lungs receive all of cardiac output so that a modest extraction ratio in the lung can contribute significantly to the clearance.

The fact still remains that the fraction of a p.o. dose reaching the general circulation is probably much greater in the dog than in the rat. In rats the bioavailability of NDMA is about 10% (12). This fact has been used to help explain the observation that chronic administration of low doses of NDMA results primarily in liver tumors whereas single high doses result in a high yield of renal tumors (1). The rationale is that at the low doses very little of the NDMA passes through the liver, but at the high doses first pass metabolism is saturated and a larger fraction of the dose reaches the systemic circulation. This concept is supported by measurements of DNA alkylation in kidney and liver following various doses of NDMA (18, 20). Given that a large fraction of a p.o. dose in dogs does pass through the liver, the organ specificity could be expected to be quite different than in the rat. The carcinogenicity of NDMA has not been tested in dogs, but chronic p.o. administration of *N*-nitrosodiethylamine resulted primarily in liver tumors (33). Since little is known about the metabolism of NDMA or *N*-nitrosodiethylamine in dogs, it is possible that the relative metabolic activities of different organs in the dog and perhaps the relative contribution of activation *versus* detoxification pathways in the dog differ substantially from those in the rat.

An interspecies comparison of the pharmacokinetics of NDMA would be interesting, but at this time adequate pharmacokinetic data are available only for rats (12), and now dogs. Some data are available for rabbits as well (13). Although the data are limited some interesting comparisons can be made. The absorption of NDMA from the gastrointestinal tract is rapid in both rats and dogs even though it has been demonstrated in rats that very little absorption occurs in the stomach (20). This can be explained from data on gastric emptying in fasted rats. In fasted rats the half-life for gastric emptying following administration of a liquid is about 12 min (34). The half-life increases to 2 h if digestible solid is given and to 4 h if undigestible solid is administered. Therefore, a dose of NDMA given by gavage to a fasted rat would enter the small intestine and be absorbed rapidly. The best available data on gastric emptying in dogs comes from studies in which radiolabeled

liver homogenate was given to the dogs (35). In 15 min 70% of the radiolabel remained in the stomach, and this decreased to 50% in 30 min and to 15% at 60 min. The behavior of a liquid should be very similar to that of the liver homogenate. The absorption half-life of NDMA in this study was about 12 min which is somewhat faster than the gastric emptying. Perhaps the absorption of NDMA from dog stomach is more rapid than rat stomach. This would not be terribly surprising since the anatomy of rat stomach is quite different than that of the dog.

The systemic clearance from blood in the dog was 47 ml/min/kg (at the 0.5-mg/kg i.v. dose). In the rat, Mico *et al.* (12) reported the systemic clearance 39 ml/min/kg. If one assumes that the entire dose of NDMA is absorbed from the gastrointestinal tract, that all of the metabolism takes place in the liver, and that all of the NDMA in blood is free (not bound to protein or cells), then the oral clearance is equal to the intrinsic hepatic clearance. In the dog it is possible that a portion of the clearance may be extrahepatic. If for the purpose of discussion this is ignored, then the oral clearance is 46 ml/min/kg in the dog and 467 ml/min/kg in the rat. Thus, despite a 10-fold difference in intrinsic clearance, the total systemic clearance in rats and dogs is similar.

The volume of distribution at steady state is also quite different in dogs and rats. In rats the V_{ss} is about 0.3 liter/kg and in dogs it is about 2.0 liters/kg. In most interspecies comparisons the volume of distribution of a compound remains relatively constant, and since NDMA is not bound to plasma protein and distributes evenly in animals it is surprising that the values are different.

These comparisons demonstrate one of the difficulties in extrapolation of data from one species to another; *i.e.*, the overall pharmacokinetics can be quite different. In extrapolating carcinogenicity data, other considerations such as the contribution of activation and detoxification pathways to the overall clearance of the compound; the status of repair processes, especially DNA repair; and the inherent susceptibility of the tissues to carcinogenesis are all confounding factors.

If humans are exposed to NDMA through environmental exposure (not by deliberate poisoning) the dose will be exceedingly low. Based on rat data, it would be expected that a very small fraction of the dose would pass through the liver. The ability of human liver to repair lesions such as O^6-methylguanine should be sufficient to substantially reduce the risk of liver cancer from this type of exposure (36). However, if it is generally true that in higher species the bioavailability of NDMA is high, then other organs will be exposed to high concentration of the carcinogen. There is evidence that other human tissues can metabolize NDMA (5–9), and repair of DNA lesions such as O^6-methylguanine does occur in tissues other than the liver (37, 38), but the rate of metabolism and rate of repair processes are not characterized well enough to even speculate about the risk of such exposure. Clearly, more data are needed on the pharmacokinetics of NDMA as well as other important nitrosamines such as the tobacco-specific nitrosamines in other mammalian species to develop a better understanding of species differences and to evaluate the feasibility of extrapolating pharmacokinetic data from animals to humans.

ACKNOWLEDGMENTS

The authors would like to thank Gerald Hutchings, Louis Gutzait, and Eric Chappel for technical assistance; Dr. David Jensen for providing the [^{14}C]NDMA; Kathy O'Bryan for performing the clinical chemistries; Dr. Bruce Mico for his invaluable advice and discussions, and Dr. Peter N. Magee for encouraging us to pursue these studies.

REFERENCES

1. Magee, P. N., Montesano, R., and Preussmann, R. Chemical carcinogenesis. Am. Chem. Soc. Monogr., *173:* 449–626, 1977.
2. Preussmann, R. Carcinogenic *N*-nitroso compounds and their environmental significance. Naturwissenschaften, *71:* 25–30, 1984.
3. Montesano, R., and Magee, P. N. Metabolism of dimethylnitrosamine by human liver slices *in vitro*. Nature (Lond.), *288:* 173–174, 1970.
4. Bartsch, H., Camus, H., and Malaveille, C. Comparative mutagenicity of *N*-nitrosamines in a semi-solid and in a liquid incubation system in the presence of rat or human tissue fractions. Mutat. Res., *37:* 149–162, 1976.
5. Autrup, H., Harris, C. C., Stoner, G. D., Jesudason, M. L., and Trump, B. F. Binding of chemical carcinogens to macromolecules in cultured human colon. J. Natl. Cancer Inst., *59:* 351–354, 1977.
6. Harris, C. C., Autrup, H., Stoner, G. D., McDowell, E. M., Trump, B. F., and Schafer, P. Metabolism of acyclic acid and cyclic *N*-nitrosamines in cultured human bronchi. J. Natl. Cancer Inst., *59:* 1401–1406, 1977.
7. Autrup, H., Harris, C. C., and Trump, B. F. Metabolism of acyclic and cyclic *N*-nitrosamines by cultured human colon. Proc. Soc. Exp. Biol. Med., *159:* 111–115, 1978.
8. Harris, C. C., Autrup, H., Stoner, G. D., Trump, B. F., Hillman, E., Schafer, P. W., and Jeffrey, A. M. Metabolism of benzo(*a*)pyrene, *N*-nitrosodimethylamine, and *N*-nitrosopyrrolidine and identification of the major carcinogen-DNA adducts formed in cultured human esophagus. Cancer Res., *39:* 4401–4406, 1979.
9. Autrup, H., and Stoner, G. D. Metabolism of *N*-nitrosamines by cultured human and rat esophagus. Cancer Res., *42:* 1307–1311, 1982.
10. Castonguay, A., Stoner, G. D., Schut, H. A., and Hecht, S. S. Metabolism of tobacco-specific *N*-nitrosamines by cultured human tissues. Proc. Natl. Acad. Sci. USA, *80:* 6694–6697, 1983.
11. Herron, D. C., and Shank, R. C. Methylated purines in human liver DNA after probable dimethylnitrosamine poisoning. Cancer Res., *40:* 3116–3117, 1980.
12. Mico, B. A., Swagzdis, J. E., Hu, H.S-W., Keefer, L. K., Oldfield, N. F., and Garland, W. A. Low-dose *in vivo* pharmacokinetics and deuterium isotope effect studies of *N*-nitrosodimethylamine in rats. Cancer Res., *45:* 6280–6285, 1985.
13. Swann, P. F. Metabolism of nitrosamines: observations on the effect of alcohol on nitrosamine metabolism and on human cancer. Banbury Rep., *12:* 53–68, 1982.
14. Skipper, P. L., Tomera, J. F., Wishnok, J. S., Brunengraber, H., and Tannenbaum, S. R. Pharmacokinetic model for *N*-nitrosodimethylamine based on Michaelis-Menten constants determined with the isolated perfused rat liver. Cancer Res., *43:* 4786–4790, 1983.
15. Magee, P. N. Toxic liver injury: the metabolism of dimethylnitrosamine. Biochem. J., *64:* 676–682, 1956.
16. Heath, D. F. The decomposition and toxicity of dialkylnitrosamines in rats. Biochem. J., *85:* 72–90, 1962.
17. Swann, P. F., and McLean, A. E. M. Cellular injury and carcinogenesis. The effect of a protein-free high-carbohydrate diet on the metabolism of dimethylnitrosamine in the rat. Biochem. J., *124:* 283–288, 1971.
18. Pegg, A. E. Alkylation of rat liver DNA by dimethylnitrosamine: effect of dosage on O^6-methylguanine levels. J. Natl. Cancer Inst., *58:* 681–687, 1977.
19. Diaz-Gomez, M. I., Swann, P. F., and Magee, P. N. The absorption and metabolism in rats of small oral doses of dimethylnitrosamine. Implications for the possible hazard of dimethylnitrosamine in human food. Biochem. J., *164:* 497–500, 1977.
20. Pegg, A. E., and Perry, W. Alkylation of nucleic acids and metabolism of small doses of dimethylnitrosamine in the rat. Cancer Res., *41:* 3128–3132, 1981.
21. Dedrick, R. Animal scale-up. J. Pharmacokinet. Biopharm., *1:* 435–460, 1973.
22. Weib, M., Szeigoleit, W., and Forster, W. Dependence of pharmacokinetic parameters on the body weight. Int. J. Clin. Pharmacol. Biopharm., *15:* 572–575, 1977.
23. Boxenbaum, H. Interspecies variation in liver weight, hepatic blood flow, and antipyrine intrinsic clearance: extrapolation of data to benzodiazepines and phenytoin. J. Pharmacokinet. Biopharm., *8:* 165–176, 1980.
24. Swabb, E. A., and Bonner, D. P. Prediction of aztreonam pharmacokinetics in humans based on data from animals. J. Pharmacokinet. Biopharm., *11:* 215–223, 1983.
25. Sawada, Y., Hanano, M., Sugiyama, Y., and Iga, T. Prediction of the disposition of β-lactam antibiotics in humans from pharmacokinetic parameters in animals. J. Pharmacokinet. Biopharm., *12:* 241–261, 1984.
26. Bonati, M., Latini, R., Tognoni, G., Young, J. F., and Garattini, S. Interspecies comparison of *in vivo* caffeine pharmacokinetics in man, monkey, rabbit, rat and mouse. Drug Metab. Rev., *15:* 1355–1383, 1985.
27. Mordenti, J. Pharmacokinetic scale-up: accurate prediction of human pharmacokinetic profiles from animal data. J. Pharm. Sci., *74:* 1097–1099, 1985.
28. Dutton, A. H., and Heath, D. F. The preparation of [^{14}C]dimethylamine and [^{14}C]dimethylnitrosamine. J. Chem. Soc., 1892, 1956.
29. Pylypiw, H. M., Zimmerman, F., and Harrington, G. W. Apparatus for trace determination of volatile *N*-nitrosamines in small samples. Anal. Chem., *57:* 2996–2997, 1985.
30. Gomeni, R. PHARM—An interactive graphic program for individual and population pharmacokinetic parameter estimation. Comput. Biol. Med., *14:* 25–34, 1984.

31. Gibaldi, M., and Perrier, D. Pharmacokinetics, Ed. 2. New York: Marcel Dekker, Inc., 1982.
32. Harrison, E. I., and Gibaldi, M. Physiologically based pharmacokinetic model for digoxin disposition in dogs and its preliminary application to humans. J. Pharm. Sci., *66:* 1679–1683, 1977.
33. Schmahl, D., Thomas, C., and Scheld, G. Cancerogene Wirkung von Diathylnitrosamin bein Hund. Naturwissenschaften, *51:* 466–467, 1964.
34. Holzer, P. Stimulation and inhibition of gastrointestinal propulsion induced by substance P and substance K in the rat. Br. J. Pharmacol., *86:* 305–312, 1985.
35. Hinder, R. A., and Kelley, K. A. Canine gastric emptying of solids and liquids. Am. J. Physiol., *233:* E335–E340, 1977.
36. Pegg, A. E., Roberfroid, M., von Bahr, C., Foote, R. S., Mitra, S., Bresil, H., Likhachev, A., and Montesano, R. Removal of O^6-methylguanine from DNA by human liver fractions. Proc. Natl. Acad. Sci. USA, *79:* 5162–5165, 1982.
37. Waldstein, E. A., Cao, E-H., Bender, M. A., and Setlow, R. B. Abilities of extracts of human lymphocytes to remove O^6-methylguanine from DNA. Mutat. Res., *95:* 405–416, 1982.
38. Myrnes, B., Giercksky, K-E., and Krokan, H. Interindividual variation in the activity of O^6-methylguanine-DNA methyltransferase and uracil-DNA glycosylase in human organs. Carcinogenesis (Lond.), *4:* 1565–1568, 1983.

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Pharmacokinetics of *N*-Nitrosodimethylamine in Beagles

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Cancer Res 1987;47:343-347.